GRASAS Y ACEITES 67 (1)
January–March 2016, e126
ISSN-L: 0017-3495
http://grasasyaceites.revistas.csic.es

Fe de Erratas/Erratum

The manuscript:
^{19}F NMR method for the determination of quality of virgin olive oil. *Grasas Aceites* **66** (4): e106. doi: http://dx.doi.org/10.3989/gya. 0242151.
The correct authors' address of the institution is as follow:

^{19}F NMR method for the determination of quality of virgin olive oil

L.L. Zhou[a,*], C. Li[b,*], X.C. Weng[a,*,✉], X.M. Fang[c] and Z.H. Gu[d]

[a]School of Life Sciences, Shanghai University, Shanghai 200444, China
[b]School of Environmental and Chemical Engineering, Shanghai University, Shanghai 200444, China
[c]Shanghai Entry-Exit Inspection Quarantine Bureau, Shanghai 200135, China
[d]Shanghai Food Security Office, Shanghai 200010, China

*These co-first authors contributed equally to this work.

✉Corresponding author: wxch@staff.shu.edu.cn

GRASAS Y ACEITES 66 (4)
October–December 2015, e106
ISSN-L: 0017-3495
doi: http://dx.doi.org/10.3989/gya.0242151

^{19}F NMR method for the determination of quality of virgin olive oil

L.L. Zhou[a,✉], C. Li[a,✉], X.C. Weng[a,✉], X.M. Fang[b] and Z.H. Gu[c]

[a]School of Environmental and Chemical Engineering, Shanghai 200444, China
[b]Shanghai Entry-Exit Inspection Quarantine Bureau, Shanghai 200135, China
[c]Shanghai Food Security Office, Shanghai 200010, China

✉Corresponding authors: wxch@staff.shu.edu.cn

Submitted: 12 February 2015; Accepted: 22 June 2015

SUMMARY: This paper reported a potential analytical technique based on NMR spectroscopy for the determination of quality of olive oil. The model compounds with active hydrogen, including free sterols, free aliphatic alcohols, phenolics, and free fatty acids were determined by ^{19}F NMR upon derivation with 4-fluorobenzoyl chloride. Integration of the appropriate signals of the derivatives of the compounds in the corresponding ^{19}F NMR spectrum allows for the quantification of these compounds. 37 Samples of commercial olive oil and 5 samples of other plant oils were determined by ^{19}F NMR. The amount of diglycerides and the ratio of 1,2-diglycerides to the total amount of diglycerides were analyzed to monitor whether extra virgin olive oil was adulterated with low price olive oil and other plant oils or not. The results showed that the total diglyceride content should not be higher than 2.5% and the ratio (D) of 1,2-diglycerides to total diglycerides should be higher than 0.35 for extra virgin olive oil. This method is an easier, simpler, safer, faster and more reliable technique for the determination of the quality of olive oil and can also be extended to monitoring the quality of ordinary edible oils.

KEYWORDS: *Alcohols; Diglycerides; 4-Fluorobenzoyl chloride; ^{19}F NMR; Quality of olive oil*

RESUMEN: ***Método ^{19}F RMN para determinar la calidad del aceite de oliva virgen***. En este trabajo se describe una técnica analítica basada en la espectroscopía de RMN para determinar la calidad del aceite de oliva. Los compuestos modelo con hidrógeno activo, incluyendo esteroles libres, alcoholes alifáticos libres, compuestos fenólicos, ácidos grasos libres se determinaron por ^{19}F RMN derivatizados con cloruro de 4-fluorobenzoilo. La integración de las señales apropiadas de los derivados de los compuestos en el correspondiente espectro de 19F RMN permite la cuantificación de estos compuestos. 37 muestras de aceites de oliva comerciales y 5 muestras de otros aceites vegetales se determinaron por ^{19}F RMN. La cantidad de diglicéridos y la proporción de los 1,2-diglicéridos a la cantidad total de diglicéridos se analizaron para monitorizar si el aceite de oliva virgen extra fue adulterado con aceite de oliva de bajo precio y otros aceites vegetales o no. Los resultados mostraron que el contenido de diglicéridos totales no debe ser superior a 2,5% y la relación (D) de 1,2-diglicéridos a diglicéridos totales debe ser superior a 0,35 para el aceite de oliva virgen extra. Este método es una técnica más simple, segura, fácil, rápida y más fiable para la determinación de la calidad del aceite de oliva y también se puede extender para monitorizar la calidad de los aceites comestibles ordinarios.

PALABRAS CLAVE: *Alcoholes; Calidad del aceite de oliva; Cloruro de 4-Fluorobenzoil; Diglicéridos; ^{19}F NMR*

Citation/Cómo citar este artículo: Zhou LL, Li C, Weng XC, Fang XM, Gu ZH. 2015. ^{19}F NMR method for the determination of quality of virgin olive oil. *Grasas Aceites* **66** (4): e106. doi: http://dx.doi.org/10.3989/gya.0242151.

1. INTRODUCTION

Olive oil is essential culinary oil of the Mediterranean diet. Olive oil is a genuine fruit juice with excellent nutritional, sensory and functional qualities obtained from the fruit of olive trees (Benito *et al*., 2013). Increasing evidence suggests that olive oil as a food has been associated with a lower incidence of chronic diseases, particularly coronary heart disease, a lower risk of cancer and other bioactive properties including anti-inflammatory, antioxidant, antiarrhythmic and vasodilatory effects (Covas Mi *et al*., 2006; López-Miranda *et al*., 2010). Many of the properties and reported health benefits of olive oil have been attributed to its minor components, such as sterols (β-sitosterol, Δ-5-avenasterol, etc), phenols (α-tocopherol, tyrosol, hydroxytyrosol, etc) and fatty acid composition (Hatzakis *et al*., 2010; Medeiros, 2001; Visioli *et al*., 2005).

Different categories of olive oil are legally defined by the European Community Regulation (EC, 2001), and are marked with different prices (Maggio *et al*., 2010). Thus, in order to increase profits there is the possibility of mixing seed oil or low-quality olive oil such as refined olive oil (ROO) and pomace olive oil (POO) with the highest quality product, extra virgin olive oil (EVOO). Besides infringing the benefits of consumers, the adulteration of extra virgin olive oil may cause serious safety problems. Olive oil adulteration resulted in over 20,000 people becoming sick and over 400 people died in 1981. This incident is called "Spanish toxic oil syndrome" (Jimeno, 1982; Tay *et al*., 2002). With economic growth, people in China are consuming more and more EVOO. At the same time, consumers and governments in China have paid a lot of attention to its quality issues. For the past 10 years the percentages of diacylglycerols (DGs) in olive oil have been investigated for their usefulness as a marker of possible adulteration (Pérez-Camino *et al*., 2001). Several studies suggest that the content of the percentages of total DGs and the ratio of 1,2-DGs to the total amount of DGs are useful indices to assess the freshness and quality of olive oil (Fronimaki *et al*., 2002; Pérez-Camino *et al*., 2001; Sacchi *et al*., 1991).

Over the past few years, NMR spectroscopy has been widely used for the analysis of virgin olive oil to evaluate quality and authentication. In particular, ^{1}H and ^{13}C NMR spectroscopies have been successfully applied to the study of minor components in virgin olive oil, and have revealed how these components are related to oil quality (Sacchi *et al*., 1996; Sacchi *et al*., 1997). ^{31}P NMR spectroscopy is a convenient method which was introduced to supplement ^{1}H and ^{13}C NMR spectrocopies, especially in cases where overlapped peaks in the ^{1}H NMR spectra or long relaxation times of the insensitive ^{13}C nuclei make the analysis a difficult task (Spyros and Dais, 2000). The ^{31}P NMR spectrum of virgin olive oil can illustrate the region of the phosphitylated total sterols, monoglycerides, DGs and free fatty acids (Vigli *et al*., 2003) by applying a method which is very fast, easy and clear, and can determine the quality of olive oil effectively.

As early as a few decades ago, a number of ^{19}F NMR reagents have been studied to readily react with various active hydrogen functional groups. Among these reagents, p-fluorobenzoyl chloride has ever been used to characterize alcohols, phenols, carboxylic acids, amines, or thiols qualitatively by fluorine-19 nuclear magnetic resonance spectrometry (Spratt *et al.*, 1984).

In this paper, we developed a completely new application of ^{19}F NMR spectra for determining the quality of olive oil because the deriving reagent for ^{31}P NMR, phosphorus trichloride, has been banned in China since 2008 due to its high toxicity. The primary task of this work was to test this safer technique with certain model compounds bearing labile protons and to determine these compounds quantitatively, then apply it to the determination and analysis of olive oil samples.

2. MATERIALS AND METHODS

2.1. Materials

All solvents and model compounds used were of reagent or analytical grade. The model compounds, stearic acid, palmitic acid, oleic acid, linoleic acid, isovaleric acid, tyrosol (98%), cholesterol, hexadecanol, dodecanol and cyclohexanol were purchased from Sinopharm Chemical Reagent Co (SHH, CHN). Hexafluorobenzene (99%) was purchased from Alfa Aesar (TJ, CHN). Pyridine and α-tocopherol were purchased from Sigma-Aldrich (SHH, CHN). All these compounds (with the exception of hexafuorobenzene and α-tocopherol) were purified by re-crystallization or distillation before use. The deriving reagent (4-fluorobenzoyl chloride) (98%) and 4-tert-butylphenol were purchased from Sigma-Aldrich (SHH, CHN). 1,3-Stearin (DG) was synthesized from glycerol and stearic acid catalyzed by Lipozyme RM IM in the laboratory according to the method used by Berger *et al.* (1992). 1,2-Stearin was made available from the partial hydrolysis of triglycerides through pancreatic lipase and separation of the formed mono- and diglycerides using thin-layer chromatography (IUPAC, 1987). 25 Olive oil samples were provided by Shanghai Entry-Exit Inspection Quarantine Bureau, and other olive oils and plant oils were purchased from a supermarket in Shanghai. Samples 1 to 24 are labeled as extra virgin olive oils. Samples 24 to 37 are labeled as blended olive oils or refined pomace olive oils.

2.2. Sample preparation

A stock solution (100 mL) composed of pyridine and $CDCl_3$ in 1:1.5 volume ratio containing ~ 0.1 mL hexafluorobenzene was prepared. Hexafluorobenzene was used as a reference for the chemical shifts. For model compounds or oil quantification purposes, 1.5 g or 0.3 g of 4-tert-butylphenol, as internal standard, were introduced in the stock solution. A predetermined quantity of the model compound (0.1 mmol) or 400 mg of olive oil were mixed with the stock solution (2 mL) in a 4 mL centrifuge tube. The required volumes of the mixed solution (0.4 mL) and the deriving reagent (~30 μL) were added in a 5 mm NMR tube. The reaction mixture was left in the NMR tube to react for about 0.5 h at room temperature. Upon completion of the reaction, the solution was examined immediately to obtain the ^{19}F NMR spectra.

2.3. NMR experiments

^{19}F NMR spectra were obtained on a Bruker Avance 500MHz spectrometer (Bern, Switzerland) operating at 470 MHz for the fluorine-19 nucleus. The probe temperature was 26 °C. Typical spectral parameters for this study were as follows: 90° pulse width, 19.3 μs; spectral width, 212 ppm; relaxation delay, 1 s; data size, 64 K (zero-filled to 128 K). For each spectrum 16 transients were acquired. Data were processed with a 0.3 Hz exponential line broadening prior to Fourier transform. Polynomial fifth-order base-line correction was performed before integration. All chemical shifts reported in this paper are relative to hexafluorobenzene in pyridine/$CDCl_3$ at δ −164.90 ppm.

3. RESULTS AND DISCUSSION

This method is based on the derivatization of the labile hydrogens of groups, such as OH and COOH, of different types of compounds with 4-fluorobenzoyl chloride according to the reaction shown in Fig. 1 and the integration of the appropriate peaks in the ^{19}F NMR spectrum.

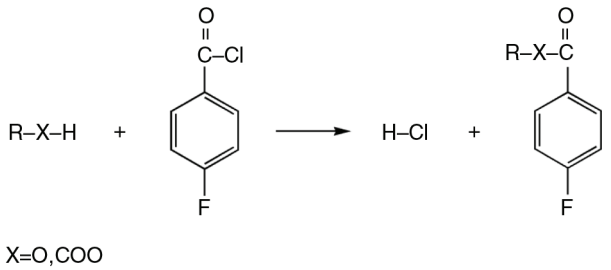

FIGURE 1. Reaction of the active hydrogen of compounds with 4-fluorobenzoyl chloride.

The natural abundance of ^{19}F is as the same as ^{31}P (100%), much higher than ^{13}C (1.11%), and a little higher than ^{1}H (99.985%), so ^{19}F NMR is sensitive and can give strong signals. The applicability of this method to the qualitative determination of these model compounds bearing active functional groups is demonstrated in Fig. 2, which illustrates the ^{19}F NMR spectrum of acids, a mixture of some model compounds, glycerol, 1,3-DGs and 1,2-DGs. Well-separated peaks for these model compounds are observed. The peak at δ −164.90 ppm and a peak at δ −107.47 ppm belong to hexafluorobenzene and the internal standard 4-tert-butylphenol, respectively. In the ^{19}F NMR spectrum (Fig. 2a), the intense peak K at δ −105.05 ppm is attributed to the deriving reagent. The broader, but smaller peak J at δ −103.57 ppm is attributed to the intermediate product in the reaction between pyridine and the deriving reagent. It does clearly show that two groups of peaks which belong to the derivative of glycerol separate very well (Fig. 2c). The α- and β-hydroxyl groups of glycerol are located at −107.89 ppm and −107.71 ppm, respectively. Moreover, the integral area of the α-hydroxyl groups is exactly twice the β-hydroxyl group.

Table 1 summarizes the ^{19}F NMR chemical shifts in the derivatives of the model compounds used in this study. Table 1 reveals a number of interesting features. First, the aliphatic hydroxyl groups are clearly distinguishable from the aromatic and other enolic hydroxyl groups in the spectra of the derivatives of the model compounds. The chemical shifts of the two derived hydroxyl groups of tyrosol can be the best illustration. The derived phenolic hydroxyl of tyrosol appears at δ −107.32 ppm and that of the aliphatic one at δ −108.58 ppm. The integral areas of derived aliphatic hydroxyl and derived phenolic hydroxyl of tyrosol are equal, which means that the aliphatic hydroxyl group and phenolic hydroxyl group can both equally react with 4-fluorobenzoyl chloride. Second, the ^{19}F chemical shifts of these model compounds containing –COOH and -OH are located in different fields. Obviously, the chemical shift of the derived carboxylic acid used in this study appears in a lower field than that of the other model compounds. Third, the ^{19}F chemical shift is sensitive to the position of the hydroxyl in 1,3-DG and 1,2-DG. Based on the chemical shifts of acids and alcohols in Table 1, it was found that several common fatty acids have the same ^{19}F NMR chemical shift, and so do several common fatty alcohols. It can be inferred that the acids and alcohols containing secondary carbons have the same chemical shifts. In our study, we found ethanol appeared to be at δ −108.93 ppm, the same as aliphatic alcohols, while isopropanol appeared to be at δ −109.13 ppm the same as cyclohexanol, which can well demonstrate the above inference. In conclusion, the relatively small size of the fluorine nucleus

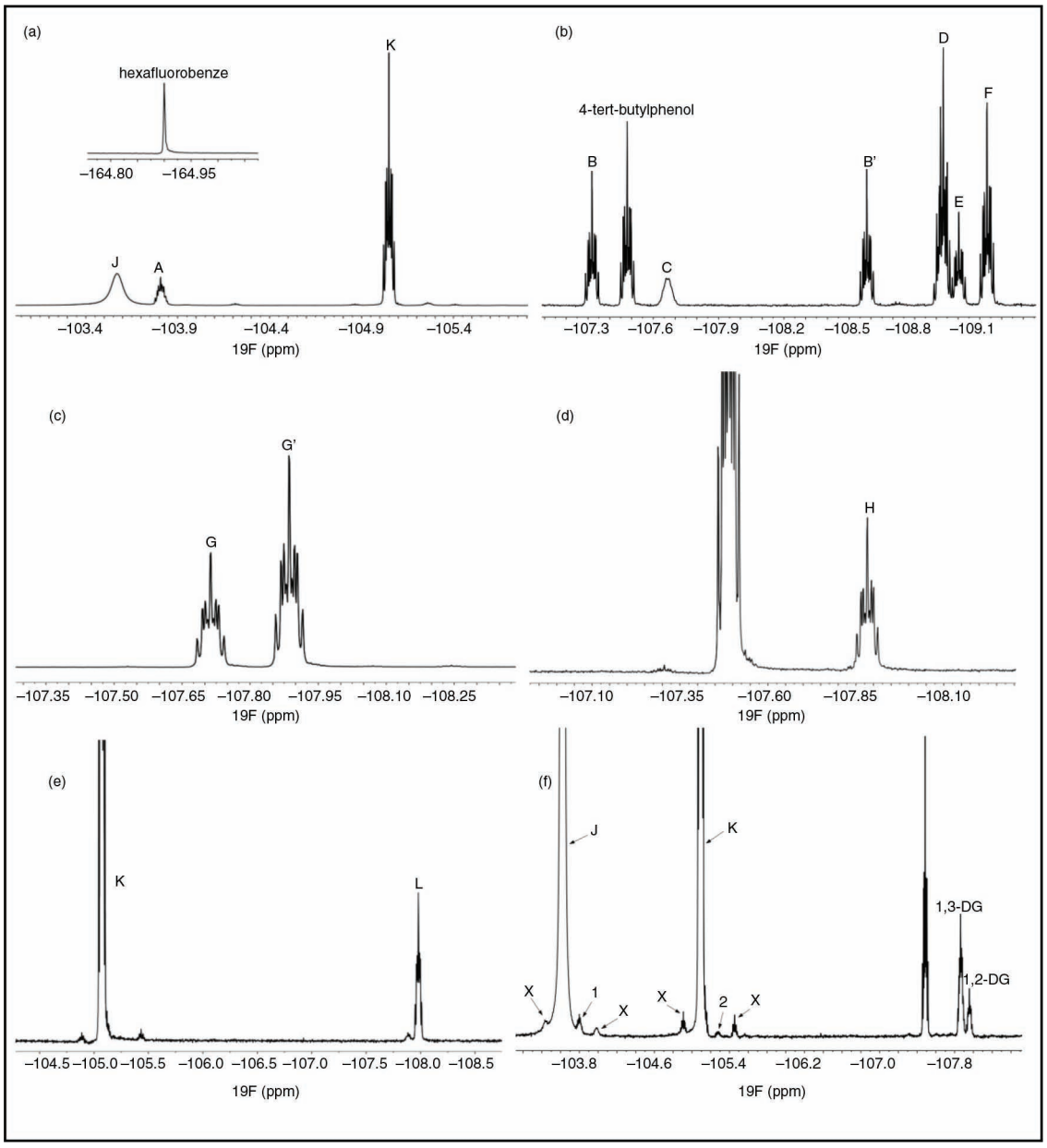

FIGURE 2. 470 MHz ^{19}F NMR spectrum of (a) fatty acids, (b) a mixture of tyrosol, α-tocopherol, hexadecyl alcohol, cholesterol and cyclohexanol, (c) glycerol, (d) 1,3-distearin, (e) 1,2-distearin and (f) an olive oil sample.

(between those of hydrogen and oxygen), a nuclear spin of 1/2, 100% natural abundance, high NMR sensitivity (83% of that of a proton) and pronounced long-range effects (Gakh *et al*., 2000) facilitate a clear distinction of these model compounds.

It was necessary to test the ability of ^{19}F NMR for quantitative analysis. The internal standard 4-tert-butylphenol was used for the quantification of various compounds. A comparison of the NMR data with a known amount of the typical compounds at different ratios for ^{19}F NMR is shown in Table 2. As can be seen, the values of relative tolerance range from 0.5 to 6.5%. Particularly, the relative tolerance for the compound of hexadecyl alcohol is the smallest, indicating that hexadecyl alcohol reacts more easily and quantitatively with the deriving reagent (4-fluorobenzoyl chloride) than other typical compounds.

Checking and comparing the ^{19}F NMR spectra of glycerol and tyrosol, primary alcohol, secondary alcohol and phenols without hindered substituents on 2,6-positions can quantitatively react with the

TABLE 1. ^{19}F NMR chemical shifts (parts per million) of derivatives of model compounds.

Signal	Compound	δ
A	stearic acid	−103.81
	palmitic acid	−103.81
	oleic acid	−103.81
	linoleic acid	−103.81
	isovaleric acid	−103.81
B	tyrosol	−107.32 (phenolic)
B'		−108.58 (aliphatic)
C	α-tocopherol	−107.66
D	hexadecanol	−108.93
	dodecanol	−108.93
E	cholesterol	−109.00
F	cyclohexanol	−109.13
G	glycerol	−107.71(β-hydroxyl)
G'		−107.89(α-hydroxyl)
H	1,3-distearin	−107.86
L	1,2-distearin	−107.95

deriving reagent. In our research, fatty acids were not shown to react with the deriving reagent quantitatively like alcohol.

37 Commercial olive oil samples from different countries and 5 other plant oil (PO) samples purchased from the local supermarket were derived with 4-fluorobenzoyl chloride, and their ^{19}F NMR spectra were recorded. A typical spectrum is shown in Fig. 2f. In the ^{19}F NMR spectrum, the two more intense peaks at δ −107.86 ppm and −107.95 ppm are attributed to 1,3- and 1,2-DGs, respectively. The peaks with Arabic numbers in the spectra have been assigned as follow: 1, free fatty acids; 2, unknown. 4-Tert-butylphenol was apparently a suitable internal standard because of its peak being well-separated from other components of olive oil and rapid reaction under conditions with the deriving reagent. The peaks, Xes appear at the same chemical shifts in the blank sample and other oil samples (Fig. 2f), which are impurities of the deriving reagent.

The integrals of these peaks are used to determine 1,2-DGs, 1,3-DGs, total DG content, and ratio D (1,2-DGs/total DGs) quantitatively. The results of such an analysis for the virgin olive oil from various countries and some plant oils are summarized in Table 3. The total amount of DGs in all VOO samples ranges from ~1.5 to ~9%. EVOO samples and blended olive oil (BOO) samples show an average mean value of total DGs of 2.51% and 5.67%, respectively. The value of total DGs of the EVOO samples is similar to Fragaki *et al*. (2005). Obviously, the total DG content in the EVOO samples is lower than that in the BOO samples which agrees with the statement that total DG concentration increases during the storage of olive oil (Fronimaki *et al*., 2002). The majority of the samples appear to contain a higher content of 1,3-DGs than 1,2-DGs which is contrary to Fragaki *et al*. (2005). Only sample 2 from Spain appears to have a lower amount of 1,3-DGs. The contents of total DGs and the ratios (D) of 1,2-DGs to the total amount of DGs are two important parameters to differentiate the olive oil samples. Previous research on the ratio (D) of virgin olive oils freshly extracted from olives of normal ripeness should be close to 1 according to Vigli *et al*. (2003). The ratios D of all samples we studied are lower than 1 which can be explained by the fact that all the samples used in our work are imported and have been stored for a long time. The ratios D in all EVOO samples range from ~0.25 to ~0.5. The EVOO samples from Spain show the highest ratios, followed by Italy and Greece. The ratios D of BOO samples are commonly lower than 0.3. For virgin olive oil, the amount of DGs and the ratio D depend on several factors, such as the olive variety, the ripeness of the olive fruit, the environment, and the storage life of the product (Fronimaki *et al*., 2002). As a result,

TABLE 2. Comparison of the measured amounts (mg) of model compounds obtained from the integration of the corresponding ^{19}F NMR signals to the weighed amounts.

Compound	Weighed	Ratio[a]	Measured[b]	Ratio[c]	R[d]
hexadecyl alcohol	193	1:4	194	1:4.01	0.5
	96	1:2	95	1:1.96	1.0
	48	1:1	47	1:0.98	2.0
cholesterol	155	1:2	161	1:2.09	3.9
	77	1:1	82	1:1.06	6.5
stearic acid	57	1:1	56	0.98	1.7
1,3-stearin (diglycerides)	125	1:1	124	0.99	0.8

[a]The mole ratio of the internal standard and the model compound.
[b]Three to four ^{19}F NMR experiments were carried out for each compound.
[c]The measured mole ratio of peak areas by ^{19}F NMR.
[d]Relative tolerance (%).

TABLE 3. Extra virgin olive oil (EVOO), blended olive oil (BOO) and some plant oils (PO): 1,2-DGs (%), 1,3-DGs (%), total DGs content (%), ratio D (1,2-DGs to the total amount of DGs).

Samples[a]	Country	1,2-DGs	1,3-DGs	Total DGs	D
EVOO					
1	Spain	0.70±0.003	1.04±0.003	1.74±0.004	0.40±0.003
2	Spain	0.76±0.039	0.76±0.005	1.52±0.048	0.50±0.010
3	Spain	0.71±0.016	1.11±0.012	1.83±0.040	0.39±0.001
4	Spain	0.77±0.039	0.99±0.024	1.74±0.087	0.44±0.001
5	Spain	0.77±0.011	0.81±0.009	1.59±0.007	0.49±0.009
6	Spain	0.68±0.003	1.79±0.044	2.44±0.084	0.28±0.011
7	Italy	0.74±0.014	1.46±0.010	2.19±0.034	0.34±0.001
8	Italy	0.71±0.031	0.93±0.003	1.65±0.024	0.43±0.012
9	Italy	0.84±0.034	1.74±0.008	2.57±0.050	0.33±0.007
10	Italy	0.92±0.015	0.89±0.010	1.83±0.005	0.51±0.010
11	Italy	0.90±0.033	1.49±0.012	2.39±0.057	0.38±0.005
12	Italy	0.68±0.003	1.95±0.033	2.61±0.069	0.26±0.006
13	Italy	0.61±0.005	1.53±0.024	2.12±0.053	0.29±0.005
14	Greece	0.68±0.017	1.43±0.006	2.11±0.029	0.32±0.004
15	Greece	0.80±0.016	2.18±0.022	2.97±0.060	0.27±0.001
16	Greece	2.06±0.002	4.65±0.053	6.68±0.109	0.31±0.005
17	Greece	0.80±0.004	2.26±0.012	3.05±0.019	0.26±0.003
18	Greece	0.64±0.015	1.51±0.008	2.15±0.032	0.30±0.002
19	Tunisia	0.77±0.002	2.15±0.033	2.89±0.069	0.26±0.006
20	Tunisia	0.84±0.044	2.32±0.022	3.15±0.001	0.27±0.014
21	Tunisia	0.83±0.019	2.17±0.025	2.98±0.069	0.28±0.001
22	Turkey	0.76±0.023	1.94±0.009	2.69±0.004	0.28±0.008
23	Turkey	0.83±0.039	2.29±0.011	3.13±0.060	0.27±0.007
24	Turkey	0.63±0.002	1.67±0.003	2.3±0.0030	0.27±0.001
BOO					
25	Italy	1.29±0.018	3.08±0.022	4.38±0.062	0.29±0.001
26	Italy	1.70±0.057	4.44±0.139	6.04±0.335	0.28±0.006
27	Italy	1.36±0.025	3.80±0.035	5.19±0.095	0.26±0.001
28	Italy	1.42±0.039	4.15±0.168	5.45±0.374	0.26±0.011
29	Italy	1.69±0.037	4.25±0.107	5.86±0.250	0.29±0.006
30	Italy	1.14±0.008	2.75±0.081	3.84±0.170	0.30±0.011
31	Spain	2.60±0.003	6.20±0.076	8.85±0.148	0.29±0.005
32	Spain	0.88±0.002	2.64±0.027	3.5±0.0530	0.25±0.004
33	Spain	2.28±0.005	5.97±0.072	8.19±0.148	0.28±0.004
34	Spain	0.79±0.015	2.19±0.004	2.98±0.007	0.27±0.005
35	Spain	2.74±0.131	6.23±0.009	8.97±0.149	0.31±0.010
36	Spain	1.27±0.014	3.34±0.015	4.59±0.016	0.28±0.004
37	Turkey	1.62±0.006	4.21±0.012	5.82±0.019	0.28±0.002
PO					
sesame oil	China	0.95±0.026	2.45±0.030	3.42±0.086	0.28±0.001
soybean oil	China	0.63±0.015	1.81±0.037	2.41±0.088	0.26±0.003
canola oil	China	0.66±0.013	1.95±0.033	2.58±0.054	0.25±0.010
camellia seed oil-1	China	0.47±0.045	1.11±0.040	1.55±0.124	0.31±0.004
camellia seed oil-2	China	0.91±0.031	2.17±0.063	3.04±0.157	0.30±0.005

[a]Data were expressed as mean ± standard deviation (n=6).

the analysis of the amount of DGs and the ratio D can be used to monitor different grades of olive oil. In addition, the 1,3-DGs content of all the five plant oils is higher than 1,2-DGs and the ratio D is ~0.3 which are in agreement with Vigli *et al*. (2003).

Figure 3 shows the ratio D against the total DGs for the samples of the extra virgin olive oils and the blended olive oils from different countries. Eight extra virgin olive oil samples (samples 1,2,3,4,5,8,10,11) are clearly gathered at the upper left of the plot (high D values and low total DGs), indicating that they are high-quality olive oil. According to EEC Standard the free fatty acid (FFA) content cannot exceed 0.8%. In theory, one mol DG (based on diolein molecular weight of 621) will produce one mol FFA (based on oleic acid molecular weight of 282) if one mol triglyceride is hydrolyzed, so 0.8% FFA will produce 1.8% total DGs. If other factors are considered, 2.5% DGs is a reasonable parameter. The other 16 samples labeled as extra virgin olive oil with lower ratio D values (<0.35) or higher total DGs content (>2.50%) tend to fall in the lower left corner, a fact that can be explained by long shelf life or adulteration with refined olive oil or even other refined vegetable oils. The blended olive oil samples (low D value and high total DGs) tend to cluster at the lower right of the plot (Fig. 3). Interestingly, the position of sample 16 falls in the same region of the plot with the BOO samples. The lower limit of the ratio D value and the upper limit of total DGs in extra virgin olive oil are 0.35 and 2.50%, respectively. As a result, it can be deduced that the samples at the upper left corner of the plot can be classified as extra virgin olive oils while the samples at the lower right corner of the plot can be classified as blended olive oils or refined olive oils.

Comparing the other 5 plant oils with olive oils, two of them have a lower DG content than 2.5%, but all of them have lower D values than 0.35. Nowadays,

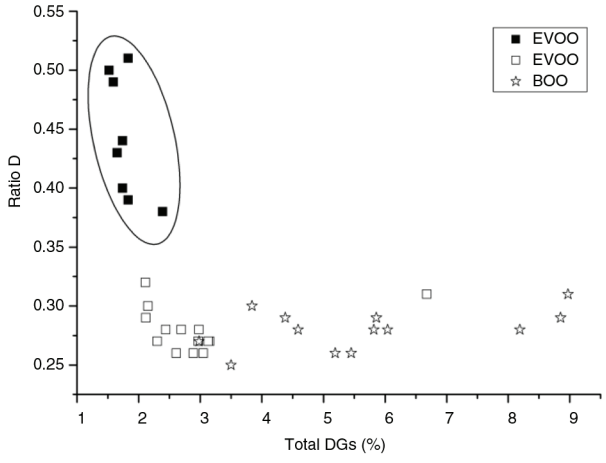

FIGURE 3. Plot of the ratio D against the total DGs for commercial extra virgin olive oils and commercial blended olive oils of different countries.

most EVOOs are adulterated with refined olive oils. If other plant oils, with the exception of tea seed oil, are added to olive oils, their oleic acid content will reduce and can be easily detected by GC or ^{1}H NMR.

4. CONCLUSIONS

The study demonstrates that the ^{19}F NMR technique has great potential to detect and analyze the quality of virgin olive oil. Its high sensitivity, excellent resolution and short detection time (~5 min) allow it to determine more minor components in olive oil. Moreover, no decomposition of products is observed for several hours at room temperature after derivatization. This method is an easier, simpler, faster, safer and more reliable technique for comparing the classic analytic methods such as HPLC, GC and chemical analysis for the determination of the quality of olive oil. This method can also be extended to monitoring the quality of ordinary edible oils. Further study is in progress in our laboratory.

ACKNOWLEDGMENTS

This work was funded by the Shanghai Food Security Office. NMR experiments were performed at the Instrumental Analysis and Research Center of Shanghai University. We thank Dr. Hong-Mei Deng for NMR spectra recording and technical assistance. The authors thank Prof. Jian Hao, Bin Xu and Wei-Guo Cao of chemistry department of Shanghai University for scientific advice.

REFERENCES

Benito M, Lasa JM, Gracia P, Oria R, Abenoza M, Varona L, Sánchez-Gimeno AC. 2013. Olive oil quality and ripening in super-high-density Arbequina orchard. *J. Sci. Food Agric.* **93**, 2207–2220. http://dx.doi.org/10.1002/jsfa.6028.

Berger M, Laumen K, Schneider MP. 1992. Enzymatic Esterification of Glycerol I. Lipase-Catalyzed Synthesis of Regioisomerically Pure 1,3-sn-Diacylglycerols. *J. Am. Oil. Chem. Soc.* **69**, 955–960. http://dx.doi.org/10.1007/BF02541058.

Covas M, Ruiz-Gutiérrez V, de la Torre R, Kafatos A, Lamuela-Raventós RM, Osada J, Owen RW, Visioli F. 2006. Minor components of olive oil: evidence to date of health benefits in humans. *Nutr. Rev*. **64**, s20–s30. http://dx.doi.org/10.1111/j.1753-4887.2006.tb00260.x.

European Community, Commission Regulation. 2001. No. 1513/2001 July 23 amending Regulations No. 136/66/EEC and No. 1638/98 as regards the extension of the period of validity of the aid scheme and the quality strategy for olive oil. Official Journal of European Communities, L201, 4–7.

Fragaki G, Spyros A, Siragakis G, Salivaras E, Dais P. 2005. Detection of Extra Virgin Olive Oil Adulteration with Lampante Olive Oil and Refined Olive Oil Using Nuclear Magnetic Resonance Spectroscopy and Multivariate Statistical Analysis. *J. Agric. Food. Chem.* **53**, 2810–2816. http://dx.doi.org/10.1021/jf040279t.

Fronimaki P, Spyros A, Christophoridou S, Dais P. 2002. Determination of the Diglyceride Content in Greek Virgin Olive Oils and Some Commercial Olive Oils by Employing ^{31}P NMR Spectroscopy. *J. Agric. Food. Chem.* **50**, 2207–2213. http://dx.doi.org/10.1021/jf011380q.

Gakh YG, Gakh AA, Gronenborn AM. 2000. Fluorine as an NMR probe for structural studies of chemical and biological systems. *Magn. Reson. Chem.* **38**, 551–558.

Hatzakis E, Dagounakis G, Agiomyrgianaki A, Dais P. 2010. A facile NMR method for the quantification of total, free and esterified sterols in virgin olive oil. *Food Chem.* **122**, 346–352. http://dx.doi.org/10.1016/j.foodchem.2010.02.043.

Paquot C, Havtfenne A. 1987. Standard methods for analysis of oils, fats and derivatives. International Union of Pure and Applied Chemistry IUPAC.

Jimeno SA. 1982. The Spanish toxic symptoms. Trends Anal. Chem. **1**, 4–6.

López-Miranda J, Pérez-Jiménez F, Ros E, De Caterina R, Badimón L, Covas MI, et al. 2010. Olive oil and health: summary of the II international conference on olive oil and health consensus report, Jaén and Córdoba (Spain) 2008. *Nutr Metab Cardiovasc Dis*. **20**, 284–294. http://dx.doi.org/10.1016/j.numecd.2009.12.007.

Maggio RM, Cerretani L, Chiavaro E, Kaufman TS, Bendini A. 2010. A novel chemometric strategy for the estimation of extra virgin olive oil adulteration with edible oils. *Food Control*. **21**, 890–895. http://dx.doi.org/10.1016/j.foodcont.2009.12.006.

Medeiros MD. 2001. Olive oil and health benefits, In R.E.C. Wildman (Ed.) Handbook of Nutraceuticals and Functional Foods. USA: CRC press, Boca Raton, 261–267.

Pérez-Camino MC, Moreda W, Cert A. 2001. Effects of Olive Fruit Quality and Oil Storage Practices on the Diacylglycerol Content of Virgin Olive Oils. *J. Agric. Food. Chem*. **49**, 699–704. http://dx.doi.org/10.1021/jf001064w.

Sacchi R, Addeo F, Paolillo L. 1997. ^{1}H and ^{13}C NMR of Virgin Olive Oil. An Overview. *Magn. Reson. Chem.* **35**, S133–S145.

Sacchi R, Paolillo L, Giudicianni I, Addeo F. 1991. Rapid ^{1}H-NMR determination of 1,2 and 1,3 diglycerides in virgin olive oils. *Ital J Food Sci*. **3**, 253–262.

Sacchi R, Patumi M, Fontanazza G, Barone P, Fiordiponti P, Mannina L, Rossi E, Segre AL. 1996. A High-Field ^{1}H Nuclear Magnetic Resonance Study of the Minor Components in Virgin Olive Oils. *J. Am. Oil. Chem. Soc*. **73**,747–758. http://dx.doi.org/10.1007/BF02517951.

Spratt MP, Don HC. 1984. p-Fluorobenzoyl Chloride for Characterization of Active Hydrogen Functional Groups by Fluorine- 19 Nuclear Magnetic Resonance Spectrometry. *Anal Chem*. **56**, 2038–2043. http://dx.doi.org/10.1021/ac00276a014.

Spyros A, Dais P. 2000. Application of ^{31}P NMR Spectroscopy in Food Analysis. 1. Quantitative Determination of the Mono- and Diglyceride Composition of Olive Oils. *J. Agric. Food. Chem*. **48**, 802–805. http://dx.doi.org/10.1021/jf9910990.

Tay A, Singh RK, Krishnan SS, Gore JP. 2002. Authentication of Olive Oil Adulterated with Vegetable Oils Using Fourier Transform Infrared Spectroscopy. *LWT- Food Sci Technol*. **35**, 99–103.

Vigli G, Philippidis A, Spyros A, Dais P. 2003. Classification of Edible Oils by Employing ^{31}P and ^{1}H NMR Spectroscopy in Combination with Multivariate Statistical Analysis. A Proposal for the Detection of Seed Oil Adulteration in Virgin Olive Oils. *J. Agric. Food. Chem*. **51**, 5715–5722. http://dx.doi.org/10.1021/jf030100z.

Visioli F, Caruso D, Grande S, Bosisio R, Villa M, Galli G, Sirtori C, Galli C. 2005. Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. *Eur J Nutr.* **44**, 121–127. http://dx.doi.org/10.1007/s00394-004-0504-0.